C1 cont

cells or in *E. coli*. The fusion proteins produced in *E. coli* are in frame with a C- or N-terminal 6 x histidine tag (noted C-His tag and N-His tag, respectively).

Please amend the specification at page 45, line 31 to page 46, line 2, as follows:

C2

Examples of available suitable antibodies to the fusion proteins of the invention include, but are not limited to, the 8029K rabbit polyclonal antibody, the mouse monoclonal c-neu-3 antibody (Calbiochem), and the mouse monoclonal HERCEPTIN® Her-2/neu antibody (U.S. Patent 5,677,171). The monoclonal c-neu-3 antibody recognizes a sequential epitope in the PD domain which is deleted (1242-1255 aa) in the ECD-ΔPD construct. The HERCEPTIN® Her-2/neu antibody binds to a conformational epitope in the ECD domain.

Please amend the specification on page 73, lines 21-25, as follows:

C3

The *E. coli* produced hECD-PD.$C_T$his was then purified on a monoQ column, and refolded in 20 mM Tris-HCL (pH 8.0) buffer. The refolded protein was tested and found to be positive for HERCEPTIN® binding by Western blot and ELISA (Fig. 17). The HERCEPTIN® Her-2/neu antibody binding activity was, however, lost later on, probably due to denaturation of the protein.

Please amend the specification on page 74, lines 3-6 as follows:

C4

The *E. coli* derived unpurified $N_T$his-hECD-PD fusion protein was recognized by the mouse c-neu-3 antibody and by a rabbit anti-ECD antibody. Following purification, the *E. coli* derived $N_T$his-hECD-PD was recognized by HERCEPTIN® Her-2/neu antibody both in Western blots and in ELISA assays (Fig. 17).

Please amend the specification on page 76, lines 28-33 as follows:

In the cell-free supernatants, secretion of full-length ECD-PD recombinant protein was very weak and only detected on Western Blots using the c-neu-3 mouse antibody (Calbiochem). Secretion and accumulation (maximum after 72 hours) of a + 70kDa protein was visible on Silver stained SDS-PAGE and detected on Western blot under non-reducing conditions with HERCEPTIN® Her-2/neu mouse antibody. This protein was not detected using the mouse c-neu-3 antibody or a mouse anti-histidine antibody (QIAGEN).

C5

Please amend the specification on page 78, lines 4-24, as follows:

The MSX transfectant clones were transferred 3-5 weeks after transfection into 24-well plates and the culture supernatants were harvested. Expression of the ECD-PD or ECD-ΔPD fusion proteins was tested by Western blot analysis using HERCEPTIN® Her-2/neu antibody under non reducing conditions. Expression of the ECD-PD fusion protein was detected in 18 out of 52 clones tested, while 13 out of 47 clones tested were positive for ECD-ΔPD expression. The selected clones expressing the fusion proteins were then readapted to suspension serum-free conditions. Based on the level of expression, growth and viability, 5 clones carrying the ECD-PD construct and 3 clones carrying the ECD-ΔPD construct were further evaluated and characterized. For the ECD-PD construct, clone 560 F3 showed the highest expression level.

C6

Expression was evaluated at 33°C in the presence or absence of sodium butyrate (2 mM) and of DMSO (2 %). Some of the clones were inducible by NaB or DMSO. Expression in CHO-K1 cells of ECD-PD and ECD-ΔPD was analyzed by Western blots and SDS-PAGE followed by either Silver or Coomassie staining. The HERCEPTIN® Her-2/neu antibody and the c-neu-3 mouse monoclonal antibodies, as well as the 8029K rabbit polyclonal antibody were used for Western blot analysis. Analysis of the culture supernatants from ECD-PD and ECD-ΔPD clones showed a band in Coomassie/Silver stained gels at 150 kDa and at 98 kDa, respectively. The same bands were revealed by HERCEPTIN® Her-2/neu antibody and by the 8029K polyclonal antiserum, as well as by the c-neu-3 antibody for ECD-PD only

C6 cont

(Fig. 18). The CHO-expressed HER-2/neu fusion proteins are recognized by the HERCEPTIN® Her-2/neu antibody (Fig. 18).

Please amend the specification on page 78, line 31 to page 79, line 3, as follows:

C7

Small scale production runs were carried out with the two best ECD-PD and ECD-ΔPD clones. Cells were cultured in suspension under serum-free conditions for 120 hours at 33°C in the presence of 2 mM Sodium butyrate. The expression of both fusion proteins was evaluated by Western blot using the HERCEPTIN® Her-2/neu antibody and by SDS-PAGE followed by silver staining using the Daiichi kit. Both fusion proteins were found to be expressed at +/- 100 μg/ml.

Please amend the specification on page 80, lines 6017, as follows:

C8

The purified fusion proteins were analyzed by SDS-PAGE followed by silver staining using the Daiichi kit, and by Western blot, using the 8029K rabbit polyclonal antibody or the mouse HERCEPTIN® Her-2/neu antibody. The analysis showed that the level of purity following the two purification steps was estimated at +/- 90% by densitometry (Biorad GS-700 Imaging Densitometer). The Western blot analysis showed that the monomers remained the major band all along the purification, that the level of oxydation was not increased, and that the detection of the epitope of interest was not modified by the conditions of purification, as shown by using the HERCEPTIN® Her-2/neu antibody. The total amount of each fusion protein recovered was measured using a colorimetric protein assay (DOC TCA BCA). This assays estimated that 2 and 4 mg of ECD-PD and ECD-ΔPD fusion protein, respectively, were purified from 75 ml of culture, with a level of purity of +/- 90%.

IN THE CLAIMS

Please amend claim s 93-113, and 116 as follows.

Please add new claims 117-124.